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Monitoring and risk assessment of tetracycline residues in foods of animal origin

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Abstract A total of 450 samples consisting of red meat, poultry meat, aquatic product and raw milk were collected during winter 2016 and summer 2017. 22.2% (100/450) of collected meat and raw milk samples were found to be contaminated with antibiotic residues in the initial screening using Premi®test. According to the enzyme linked immunosorbent assay (ELISA) results, the mean tetracyclines (TCs) concentration of meat samples determined as follows: chicken (155.41 µg/kg) > turkey (138.68 µg/kg) > quail (130.7 µg/kg) > cow (108.92 µg/kg) > calf (105.18 µg/kg) > goat (99.4 µg/kg) > sheep (95.22 µg/kg) > rainbow trout (35.62 µg/kg) > shrimp (31.80 µg/kg). The content of TCs in cow, goat and sheep milk samples were found to be ranged 45.6–163.5 µg/L, 72.4–101.1 µg/L and 65.5–98.9 µg/L, respectively. 5.7% (26/450) of samples had TCs confirmed the ELISA results using high performance liquid chromatography coupled with ultra-violet detection, although the concentration of TCs residues in samples was higher than that of ELISA.

Keywords Tetracycline · Risk assessment · Meat · Milk · Iran

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Introduction

Veterinary antibiotics are utilized in animal husbandry, aquaculture and apiculture activities to treat and prevent various bacterial infections and enhance growth rates (Shahbazi et al., 2015). Among them, tetracyclines (TCs) including oxytetracycline (OTC), tetracycline (TC), chlortetracycline (CTC) and doxycycline (DXC) are extensively used in food producing animals owing to their low cost, availability, ease of administration and broad-spectrum antimicrobial activity (Ahmadi et al., 2015; Yu et al., 2011). According to the Food and Drug Administration (FDA), TCs showed the highest level of drug used in food producing animals (FDA, 2012). However, due to improper administration and/or insufficient withdrawal period after treatment, there are increasing concerns about the presence of their residues in milk and edible animal tissues (Feng et al., 2016; Shalaby et al., 2011). The TCs residues can directly pose toxic and dangerous effects on the consumer's health e.g. allergic reactions, drug resistance to microorganisms, poor fetal development, gastrointestinal disturbance and TC pigmentation teeth (Han et al., 2015; Mookantsa et al., 2016; Yu et al., 2011). For protection of consumer health, strict regulations for TCs have been established by the national regulatory agencies and international organizations such as Codex Alimentarius, European Commission (EC) and FDA (Ngoc Do et al., 2016). The EC has determined maximum residue levels (MRLs) of each TC derivative at 100 µg/kg, 300 µg/kg and 600 µg/kg for muscle, liver and kidney of all food-producing species, respectively (European Commission, 1995). The MRL of 100 µg/L for each TCs in milk has been prescribed by Codex Alimentarius (Codex Alimentarius Commission, 2012). The FDA has established an acceptable daily intake (ADI) for OTC, TC and CTC as 25 µg/kg bw/day (Food and Drug Administration, 2004).

Microbiological tests (Ngoc Do et al., 2016) and immunoassays (Wang et al., 2015) have been generally described for screening of TCs in food products. The most important disadvantages of microbiological tests are lack of specificity and the long required incubation (Shahbazi et al., 2015). Owing to very close structural similarity of TCs immunoassays also can result in a false-positive detection (Barani and Fallah, 2015). Therefore, a confirmatory analysis for quantification of screening test results in food products such as HPLC with various detection techniques including HPLC with mass spectrometry (MS) (Han et al., 2015), HPLC with fluorescence detection (FD) (Peres et al., 2010) and HPLC with ultra-violet detection (UV) (Ahmadi et al., 2015; Shalaby et al., 2011; Yu et al., 2011) is necessary. In spite of strict regulations for TCs, numerous studies have been conducted on the occurrence of TCs residues in food products such as chicken meat (Ahmadi et al., 2015; Salama et al., 2011; Shahbazi et al., 2015; Shalaby et al., 2011; Yu et al., 2011), egg (Feng et al., 2016), milk (Feng et al., 2016; Han et al., 2015; Mamani et al., 2009; Vragović et al., 2012; Zhang et al., 2014), beef meat (Mesgari Abbasi et al., 2011; Mookantsa et al., 2016), pork meat (Feng et al., 2016; Ngoc Do et al., 2016; Yu et al., 2011) and honey (Peres et al., 2010; Wang et al., 2015). In Iran, there is very little information about the use of TCs antibiotics in food products and the public health effects on food safety. Previous studies (Ahmadi et al., 2015; Shahbazi et al., 2015) indicated that some edible chicken tissues (liver, kidney and meat) contained TCs residues in Kermanshah province, west of Iran. It seems that a practical monitoring system for residual antibiotics should be established. Therefore, the aims of the present study were (1) screen the presence of antibiotic residues in foods of animal origin including red meat (cow, calf, goat and sheep), poultry meat (chicken, quail and turkey), aquatic product (shrimp and rainbow trout) and raw milk (cow, goat and sheep) using Premi®test kit; (2) investigate the occurrence of TCs residues in the corresponding samples using an enzyme linked immunosorbent assay (ELISA) technique; and (3) confirm the positive results by a HPLC–UV.

Materials and methods

Analytical standards

The reference substances of OTC, TC, CTC and DXC were obtained from Sigma-Aldrich (St. Louis, MO, USA). Individual standard stock solutions of antibiotics with concentration of 1 mg/mL were prepared in methanol and kept in amber flasks at 4 °C until further use.

Apparatus

HPLC analysis were conducted using a KNAUER liquid chromatography system equipped with a temperature controller (Jetstream two plus), a manual six-valve injector fitted with a 20 µL loop (7725i-made in USA) and a PDA detector (UV detector 2600 model). Separations were performed on a Eurospher RP-C18 column (250 × 4.6 mm i.d.). A guard column (Eurospher 100-5 C18) was used to protect the analytical column.

Study area and sample collection

The sampling was performed according to the methodology described by the Codex Alimentarius for monitoring program associated with the use of veterinary drugs in food producing animals (Codex Alimentarius, 2009). Three hundred and sixty (n = 360) meat samples from cow, calf, goat, sheep, chicken, quail, turkey, shrimp and rainbow trout were purchased from highly populated local markets (Kermanshah, Iran) during winter 2016 and summer 2017. Ninety samples (n = 90) of raw milk of cow, goat and sheep were also collected from different dairy farms that hold outlets of raw milk and dairy products. The obtained samples were kept in a cooler with ice for transferring to the laboratory, where these were stored at − 18 °C. The samples free of antibiotic residues as blanks were used to validate the HPLC method.

Antimicrobial residues screening assay using Premi®test

Screening assay using Premi®test kit (R-biopharm, Darmstadt, Germany) was conducted. Sample preparation and working protocol were performed according to the manufacturer's instruction.

Sample preparation

ELISA analysis

5 g of each meat sample was weighted, homogenized using a mechanical homogenizer (HG-15D, Wise Tis, Korea), to ensure complete tissue disruption. Then, 1 g of homogenized sample was added into 9 ml of PBS buffer in 15 mL falcon tube. The resulting mixture was shacked for 10 min and centrifuged at 4000×*g* for 10 min at room temperature. 1 ml of supernatant was transferred to a new falcon tube and 2 ml of hexane was added. After vortexing, the mixture was centrifuged at 4000×*g* for 10 min at room

temperature and 50 µL of lower layer was used per well for ELISA analysis (Shahbazi et al., 2015). Frozen milk samples were thawed and defatted by centrifugation at 3000×*g* at 10 °C for 10 min. 100 µL of the defatted supernatant was diluted with sample dilution buffer and then used per well in the test (Zhang et al., 2014).

HPLC–UV confirmatory analysis

1 g of each homogenized meat sample was putted into 15 mL falcon tube. Then, 1 mL of Briton Robinson buffer (0.008 M and pH 10) was added and the vortex-mixing at 600 rpm for 10 min was conducted. The sample was centrifuged at 4000×*g* using refrigerator centrifuge (sigma 3–30 k model) for 10 min at 4 °C and 100 µl of the supernatant was subjected to solid phase extraction (SPE) according to the published method by (Ahmadi et al., 2015). Milk samples were prepared based on the method of (Mamani et al., 2009) for quantification of TCs in bovine milk by HPLC–DAD.

Determination of tetracyclines using ELISA and HPLC–UV

Determination of TCs residues by ELISA was conducted according to the kit structure. All separations was performed under gradient elution with mobile phase composed with a mixture of methanol: acetonitrile: 0.03 M oxalic acid buffer: water (10:17:50:23), which kept isocratic mode for 9 min, changing to 10:23:50:17 in 7 min and then returning to original conditions in 6 min. The injection volume and flow rate were 20 µl and 0.9 ml/min, respectively. The TCs was detected by means of a UV detector at wavelength set at 353 nm (Shalaby et al., 2011).

HPLC method validation

The method was validated according to the validation procedure for antibiotic residues in food products with animal origin described by the European Community (European Communities, 2002). Validation parameters including the linearity, specificity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ) were evaluated.

Response linearity for each type of sample was evaluated by analyzing 21 blank samples fortified with standard solutions of the antibiotics at three concentration levels of 0.5, 1 and 1.5 MRL. The calibration curve of each sample was plotted using mean peak area ratio versus corresponding concentrations injected, and the equations of the curves, the coefficients of determination (r^2) and correlation (r) were determined by linear regression. Specificity of the method was checked by analyzing each type of eleven blank samples from different origins to examine the presence of any possible endogenous interferences such as signals or peaks near the retention time of the TCs compounds. Moreover, an appropriate number of blank samples were spiked with the standard solutions of investigated analytes as well as macrolides and lincosamides that are probably to interfere with the identification and quantification of the TCs. Accuracy and precision of the proposed method, reported as relative standard deviation (RSD) and recovery (%), respectively, were evaluated by analyzing 21 blank samples spiked with all the analytes at levels of 100 and 200 µg/kg. The recoveries were calculated according to the following equation: Recovery (%) = 100 × measured content/spiked level. The LOD and LOQ for the analyte was established based on the following equations (Chung et al., 2009): LOD (limit of detection) = 3 × SD/Slop and LOQ (limit of quantification) = 10 × SD/Slope.

Statistical analysis

Each sample was analyzed in triplicate. The analysis was performed using SPSS 16 (version 16; SPSS Inc, USA) for Windows software package. One way analysis of variance (ANOVA) and Dunnett's T3 (as the post hoc analysis) tests were used to compare TCs residues in different samples. Mann–Whitney U test was used to compare the results of total TCs between ELISA and HPLC–UV. Significance level was considered at $P < 0.05$ in all analyses.

Results and discussion

Antimicrobial residues screening assay using Premi® test

As shown in Table 1, 77 (21.3%) out of 360 meat samples and 23 (25.5%) out of 90 raw milk samples were positive results. Significant differences were found in the veterinary drug residues of collected samples during winter and summer seasons ($P < 0.05$). The presence of high antibiotic residues in raw milk and meat samples collected from winter is probably due to the higher prevalence of respiratory diseases and subsequently more TCs use in this season (Shahbazi et al., 2015).

Screening of tetracycline residues in meat and raw milk samples using ELISA

The occurrence of TCs residues in meat and raw milk samples are presented in Table 2. Based on the results of the present study, 19 out of 360 meat samples had antibiotic concentration levels higher than the maximum residue level (MRL) for TCs set by the European legislation

Table 1 Detection of antibiotic residues in meat and raw milk samples by Premi® test; comparison between winter and summer seasons

Sample	Summer (n = 225), n (%)	Winter (n = 225), n (%)	Total (n = 450), n (%)
Red meat			
Cow	4 (20.0)	6 (30.0)	10 (25.0)
Calf	2 (10.0)	5 (25.0)	7 (17.5)
Goat	3 (15.0)	5 (25.0)	8 (20.0)
Sheep	3 (15.0)	6 (30.0)	9 (22.5)
Poultry meat			
Chicken	4 (20.0)	8 (40.0)	12 (30.0)
Turkey	3 (15.0)	6 (30.0)	9 (22.5)
Quail	3 (15.0)	6 (30.0)	9 (22.5)
Aquatic product			
Shrimp	2 (10.0)	4 (20.0)	6 (15.0)
Rainbow trout	3 (15.0)	4 (20.0)	7 (17.5)
Raw milk			
Cow	4 (26.6)	7 (46.6)	11 (36.6)
Goat	2 (13.3)	4 (26.6)	6 (20.0)
Sheep	2 (13.3)	4 (26.6)	6 (20.0)

Table 2 Tetracyclines residues (µg/kg or µg/L) in the meat and raw milk samples determined by ELISA

Sample	Sample tested, n	Positive samples		
		Mean ± SE (µg/kg or µg/L)	Min–max (µg/kg or µg/L)	Exceed regulation[a], n (%)
Red meat				
Cow	40	108.92 ± 33.0	39.91–301.1	3 (7.5)
Calf	40	105.18 ± 21.1	55.0–176.6	2 (5.0)
Goat	40	99.40 ± 5.10	87.8–116.6	2 (5.0)
Sheep	40	95.22 ± 14.0	66.5–145.2	2 (5.0)
Poultry meat				
Chicken	40	155.41 ± 40.9	54.3–389.1	4 (10.0)
Turkey	40	138.68 ± 44.6	40.9–345.1	3 (7.5)
Quail	40	130.70 ± 38.3	43.2–295.9	3 (7.5)
Aquatic product				
Shrimp	40	31.80 ± 12.7	7.6–60.8	0 (0.0)
Rainbow trout	40	35.62 ± 11.7	11.20–65.6	0 (0.0)
Raw milk				
Cow	30	107.71 ± 27.5	32.31–246.7	3 (10.0)
Goat	30	103.45 ± 36.6	27.7–202.8	2 (6.6)
Sheep	30	79.63 ± 27.0	26.63–145.5	2 (6.6)

[a]The Codex regulation limit for tetracyclines residues in foodstuffs is 200 µg/kg

(Council Regulation (EEC), 1990). The order of contaminated meat samples were as follows: chicken > turkey > quail > cow > calf > goat > sheep > rainbow trout > shrimp. The highest mean concentration of TCs were found in chicken meat (155.41 µg/kg), while shrimp by 31.80 µg/kg significantly showed the lowest concentration ($P < 0.05$).

The average level of TCs contents of chicken, quail and turkey samples were 155.41 µg/kg, 130.70 µg/kg and 138.68 µg/kg, with ranges of 54.3–389.1 µg/kg, 43.2–295.9 µg/kg and 40.9–345.1 µg/kg, respectively (Table 2). Shahbazi et al. (2015) also reported the concentration of TCs residues in chicken meat as 45–412 µg/kg. Alavi et al., (2015) reported that the mean TCs concentration in the Iranian chicken manure samples was found in the range of 160–763 µg/kg. Salama et al., (2011) also indicated the high concentration of TCs (107–6010 µg/kg) in chicken meat obtained from Egypt. Based on Regulation No 2377/90/EC of EU, the maximum residue level (MRL) for TCs in muscle tissue of all food-producing species were allowed 100 µg/kg (Council Regulation (EEC), 1990). Accordingly, about 10% (4/40), 7.5% (3/40) and 7.5% (3/40) of chicken, quail and turkey samples had TCs level that exceeded this limit. The Codex Alimentarius Commission and US FDA have set the MRL at 200 µg/kg for corresponding tissue. Therefore, the concentration level of five samples exceeding the maximum permissible limit of these commissions.

As described in Table 2, TCs residues was found in 10% (4/40) and 10% (4/40) of rainbow trout and shrimp samples with mean concentrations of 35.62 µg/kg and 31.80 µg/kg, respectively. The concentration level of none of them exceeded the maximum permissible limit of 200 µg/kg established by the Codex Alimentarius Commission (Codex Alimentarius Commission, 2011). Barani and Fallah (2015) and Vragović et al. (2012) reported that the average concentration of TCs in rainbouw trout samples obtained from Iran and Croatia were 8.98 µg/kg and 1.7 µg/kg, respectively. Swapna et al. (2012) also found that the TCs residues in farmed shrimp samples were in the ranges of 37.61–46.60 µg/kg. The most important reason for the low concentrations of TCs residues in aquatic products is probably related to this fact that fish farmers prefer the use of sulfonamides, fluoroquinolones and also florfenicol instead of TCs for management of different stages of diseases in aquatic animals (Swapna et al., 2012).

With regards to the TCs residues in red meats from four species (cow, calf, goat and sheep), 20% (mean: 108.92 µg/kg), 12.5% (mean: 105.18 µg/kg), 12.5% (mean: 99.40 µg/kg) and 12.5% (mean: 95.22 µg/kg) of cow, calf, goat and sheep were found contaminated with TCs compounds. Most of positive cow, calf, goat and sheep samples had TCs residues in the ranges of 39.91–301.1 µg/kg, 55.0–176.6 µg/kg, 87.8–116.6 µg/kg and 66.5–145.2 µg/kg, respectively (Table 2). About 7.5% (3/40), 5% (2/40), 5% (2/40) and 5% (2/40) of cow, calf, goat and sheep had TCs content that exceeded the EU limit, respectively. According to the previous studies, TCs was found to be the most important antibiotic residue in cow farms in Iran (Mesgari Abbasi et al., 2011). In another study (Javid et al., 2016), reported moderate concentrations of TCs (4.4–59.3 µg/L) in surface water resources owing to livestock production. These profiles recommend that TCs has been extensively used in livestock farming and subsequently the findings of the current study was expected. The detected percentages of TCs were higher than those of reported in Japan (Osaka Prefectural Institute of Public Health, 2012) and Vietnam (Yamaguchi et al., 2015), which revealed positive sample percentages of 0.8% and 7.4% in beef meat, respectively. In accordance with the results of this study, Mesgari Abbasi et al. (2011) reported average concentration of TCs in cattle muscle collected from Ardabil, northwest of Iran was 106.3 µg/kg.

As shown in Table 2, the order of TCs concentration (µg/L) in raw milk samples analyzed are as follows: cow > goat > sheep. TCs residues were detected in 7 (23.3%), 4 (13.3%) and 4 (13.3%) of cow, goat and sheep milk samples with concentrations ranging 32.31–246.7 µg/L, 27.7–202.8 µg/L and 26.63–145.5 µg/L, respectively. The mean concentration of TCs in the present study is in agreement with those reported in raw milk from Iran (Abbasi et al., 2011). Accordingly, the mean concentrations of TCs residues in raw milk collected from Ardabli, northwestern of Iran was 109.1 µg/L. In another study (Vragović et al., 2012), the mean concentration of the corresponding antibiotic residues in raw milk samples collected from Croatia was 99.4 µg/L.

In comparison with Premi® test, 26 suspected samples were determined as false positive, which is equal to frequency of 7.2%. This false positive rate determined by the Premi® test is in accordance with previous studies by (Pikkemaat et al., 2011) and (Ngoc Do et al., 2016). Use of Premi® test in combination with ELISA is still powerful and accurate approach for the early detection of potential hazards in foods of animal origin. Some previous studies also described the parallel application of microbial screening assays and ELISA for monitoring antibiotic residues in chicken meat (Salama et al., 2011; Shahbazi et al., 2015), raw milk (Chung et al., 2009) and pork meat (Ngoc Do et al., 2016).

Validation study

The proposed method showed to be linear throughout all the tested concentrations for each analyte with the range corresponding correlation of 0.9991–0.9999. No statistically significant differences were found for the slope and intercept among the calibration curves ($P > 0.05$). Deviations of the individual points from the calibration curve were lower than 10%. The specificity of the method was checked by analyzing each type of eleven blank samples from different origins to examine the presence of any possible endogenous interferences such as signals or peaks near the retention time of the TCs compounds. Injection of macrolides and lincosamides was also conducted in order to evaluate their potential interference with the identification and quantification of the TCs. The mean recovery values were in the range of 82–95%, 71.88–100%, 81.37–94.6% and 82–97.67% for OTC, TC, CTC and DXC, respectively. The obtained results are in accordance with the criteria (70–120%) established by the EC

Table 3 Frequency of tetracyclines residues and their concentrations detected in meat samples using HPLC

Antibiotic	Sample								
	Cow	Calf	Goat	Sheep	Chicken	Turkey	Quail	Shrimp	Rainbow trout
Oxytetracycline									
Positive samples n, (%)	3 (7.5)	2 (5)	2 (5)	2 (5)	4 (10)	3 (7.5)	2 (5)	1 (2.5)	1 (2.5)
Range (µg/kg)	77.9–352.6	95.7–281.1	91.3–225.4	78.5–221.6	67.5–425.3	89.7–355.1	88.9–300.4	68.2	70.6
Tetracycline									
Positive samples n, (%)	ND[a]	ND	ND	ND	ND	ND	ND	ND	ND
Range (µg/kg)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Chlorotetracycline									
Positive samples n, (%)	2 (5)	1 (2.5)	1 (2.5)	1 (2.5)	2 (5)	1 (2.5)	1 (2.5)	1 (2.5)	1 (2.5)
Range (µg/kg)	80.1–121.2	83.6	82.4	83.5	91.2–252.6	163.2	130.8	71.2	71.5
Doxycycline									
Positive samples n, (%)	1 (2.5)	1 (2.5)	1 (2.5)	1 (2.5)	2 (5)	1 (2.5)	1 (2.5)	ND	ND
Range (µg/kg)	101.2	78.5	80.3	77.7	103–127.6	101.6	100.6	ND	ND
Total	6 (15)	4 (10)	4 (10)	4 (10)	8 (20)	5 (15)	4 (10)	2 (5)	2 (5)

[a] *ND* not detected

(European Communities, 2002). According to the RSDs, the proposed method was suitable for four TCs studied in different types of matrices since all values were lower than 10%, which are in agreement with the reference parameters established by the EC (European Communities, 2002). LOD and LOQ of the suggested method were found to be in the ranges of 3.7–9 and 9–27 µg/kg or µg/L, respectively, suggesting that the proposed method is appropriate to determine the TCs residues in meat and raw milk samples at µg/kg or µg/L levels.

Confirmation of tetracycline residues in meat and raw milk samples using HPLC–UV

In the present study, after microbial and immunological screening assays, all positive samples were analyzed by validated HPLC–UV method. There were no significant differences in the results of total TCs between ELISA and HPLC–UV ($P > 0.05$). As presented in Table 3, 39 meat samples had TCs residues in the range of 67.5–425.3 µg/kg. TCs were found in 8.8% (8 out of 90) of raw milk samples (ranging 45.6–163.5 µg/L). Any of target antibiotics was not detected from remaining positive samples via microbial and immunological screening tests, which these should be considered as false positive. The order of TCs concentrations in meat samples are as follow: chicken (163.1 µg/kg) > turkey (159.7 µg/kg) > quail (155.17 µg/kg) > cow (142.35 µg/kg) > calf (134.72 µg/kg) > goat (119.85 µg/kg) > sheep (115.32 µg/kg) > rainbow trout (71.05 µg/kg) > shrimp (69.7 µg/kg). The content of TCs in cow, goat and sheep milk samples were found to be ranged 45.6–163.5 µg/L, 72.2–101.1 µg/L and

Table 4 Frequency of tetracyclines residues and their concentrations detected in milk samples using HPLC

Antibiotic	Sample		
	Cow	Goat	Sheep
Oxytetracycline			
Positive samples n, (%)	2 (6.6)	1 (3.3)	1 (3.3)
Range (µg/L)	80.4–163.5	101.1	98.9
Tetracycline			
Positive samples n, (%)	ND[a]	ND	ND
Range (µg/L)	ND	ND	ND
Chlorotetracycline			
Positive samples n, (%)	1 (3.3)	1 (3.3)	1 (3.3)
Range (µg/L)	75.7	72.2	65.5
Doxycycline			
Positive samples n, (%)	1 (3.3)	ND	ND
Range (µg/L)	45.6	ND	ND
Total	4 (13.3)	2 (6.6)	2 (6.6)

[a] *ND* not detected

Table 5 The estimated daily intake (EDI) and EDI/acceptable daily intake (ADI)% for tetracycline residues via consumption of foods of animal origin

	Red meat	Poultry meat	Aquatic products	Raw milk
EDI[a]				
ELISA	0.05	0.14	0.01	0.33
HPLC–UV	0.06	0.16	0.02	0.29
EDI/ADI[b] (%)				
ELISA	0.00002	0.000056	0.000004	0.000132
HPLC–UV	0.000024	0.000064	0.000008	0.000116

[a]Estimated daily intake (µg/60 kg body weight/day)

[b]Acceptable daily intake (ADI) of TCs residues via food consumption is 25 µg/60 kg body weight/day

65.5–98.9 µg/L, respectively (Table 4). The descending order concentration level of TCs is as follows: OTC > CTC > DXC. The probable reason behind this can be that that although both OTC and CTC are approved for nutrition, prophylactic and therapeutic uses, both TC and DXC should be used only as therapeutic agents in food-producing animals (Shalaby et al., 2011). The high contamination of meat samples with TCs residues especially OTC are reported by other authors from Iran (41–889 µg/kg) (Shahbazi et al., 2015) and Egypt (103–2930 µg/kg) (Salama et al., 2011).

Assessment of exposure to tetracyclines due to meat and milk consumption

Human risk assessment due to long-term exposure of TCs residues was conducted by evaluating estimated daily intake (EDI), according to the method previously described by (Shahbazi et al., 2016). Based on Annual Agricultural Statistics of Iran, the annual consumption of red meat, poultry meat, aquatic products and milk per each person has been estimated to be 11 kg, 25 kg, 9.2 kg and 70 l in 2013, respectively. In this study, mean body weight for the adult Iranian population was considered 60 kg. Acceptable daily intake (ADI) limit of TCs residues via food consumption (for a person weighing 60 kg) is 25 µg/kg bw/day (JECFA, 2003). The risk can be considered as negligible when the ''level of achievement'' was less than 1% of ADI, as considerable if ''level of achievement'' was 1–5% of ADI and as distinctively if ''level of achievement'' was greater than 5% of ADI (Vragović et al., 2012). The exposure to investigated antibiotic residues in meat and raw milk samples are presented in Table 5. The maximum EDI value was observed for raw milk. The obtained values of none of them exceeded the recommended ADI limit. There is few data in the literature regarding risk assessment of TCs due consumption of meat and milk products. According to the data for OTC in USA, EDI was 9.6 µg/person/day (US EPA, 2006). In another study, (Vragović et al., 2012) reported that the EDI of TCs through average consumption of meat in Croatia based on median value for those veterinary drugs is low (0.52 µg/person/day). Results of the present study showed that EDI of TCs could be generally considered as safe; thereby, there is no adverse health effect due to the consumption of milk and meat in Iranian consumers.

It should be taken into account that Premi® test is an appropriate approach for the early detection of potential hazards due to its low cost and simple operation. Based on the ELISA results, the average concentration of TCs in chicken, turkey, quail, cow, calf, goat, sheep, rainbow trout, shrimp and raw milks of cow, goat and sheep were 155.41 µg/kg, 138.68 µg/kg, 130.7 µg/kg, 108.92 µg/kg, 105.18 µg/kg, 99.4 µg/kg, 95.22 µg/kg, 35.62 µg/kg, 31.80 µg/kg, 107.71 µg/L, 103.45 µg/L and 79.63 ± 27.0 µg/L, respectively. Regarding HPLC results, the order of TCs concentrations in meat samples are as: chicken (163.1 µg/kg) > turkey (159.7 µg/kg) > quail (155.17 µg/kg) > cow (142.35 µg/kg) > calf (134.72 µg/kg) > goat (119.85 µg/kg) > sheep (115.32 µg/kg) > rainbow trout (71.05 µg/kg) > shrimp (69.7 µg/kg). The content of TCs in cow, goat and sheep milk samples were found to be ranged between 45.6–163.5 µg/L, 72.2–101.1 µg/L and 65.5–98.9 µg/L, respectively. These results indicate that parallel use of microbial and immunological screening methods, which are then forwarded for confirmatory chemical analysis mostly based on HPLC is one of the most powerful and accurate approach for the detection of antibiotic residues in foods of animal origin.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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